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International study on *Artemia* XLIX.† Salinity effects on survival, maturity, growth, biometrics, reproductive and lifespan characteristics of a bisexual and a parthenogenetic population of *Artemia*

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Abstract

Two *Artemia* populations, a bisexual from San Francisco Bay (California, USA) and a parthenogenetic from Tanggu area (Tianjin province, People's Republic of China) are assayed for their tolerance and fitness in various salinity levels. This study was carried out under laboratory conditions where salinity effects upon special characteristics of the two *Artemia* populations, such as survival, growth rate, maturation, morphology, fecundity and life duration, were recorded. This evaluation revealed that the two populations examined exhibit significant differences in their response against elevated salinity levels. Furthermore, specific biometric parameters can be a useful tool for the discrimination and/or determination of their distribution in a mixed population, which, in fact, is the case in Tanggu salt works. *Artemia franciscana* seems to be a more effective colonizer at higher salinities. The data presented in this study may generate useful suggestions for proper management of the solar saltworks at Tanggu although further experimentation is needed.

Introduction

The brine shrimp *Artemia* (Crustacea, Anostraca) is a genus with a wide distribution on the five continents, inhabiting inland salt lakes, coastal lagoons and solar saltworks (Vanhaecke *et al.*, 1987). It comprises a complex of sibling species and superspecies defined by the criterion of reproductive isolation (Browne & Bowen, 1991). *Artemia* is used as a source of live food in the aquaculture of freshwater and marine fish and crustacean species (Bengtson *et al.*, 1991).

One of the largest areas for solar salt production is located in the Bohai Bay, People's Republic of China (PRC), Tianjin province, where several thousands of hectares of evaporation ponds are in operation. In recent years the Bohai Bay has been more and more eutrophied, resulting in high primary production in the salt ponds. Tackaert & Sorgeloos (1991) reported that the local parthenogenetic population is characterized by limited productivity and has poor resistance to the high salinities and low temperatures that prevail in the salt ponds especially during spring. As the local *Artemia* population cannot control the algae blooms, the salt produced in the Bohai Bay saltworks has a low quality and measures were taken to remedy this industrial problem (Cheng, 1991). Tackaert & Sorgeloos (1991) initiated an inoculation project using *Artemia franciscana* from San Francisco Bay (USA) in order to improve the hydrobiological conditions at Tanggu saltworks by increasing the *Artemia* production.

† International interdisciplinary study on *Artemia* populations coordinated by the Laboratory of Aquaculture & Artemia Reference Center, University of Ghent, Belgium.

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In the present paper a comparative study between the bisexual *A. franciscana* and *A. parthenogenetica* from Tanggu is accomplished in terms of different morphological parameters, survival, growth and maturation rate under different salinities (for more details see Wear & Haslett, 1986 and Wear *et al.*, 1986). Also, the reproductive and life span characteristics of the two *Artemia* species are recorded under laboratory conditions. It is expected that these laboratory culture studies could provide insights into the potential response of the studied *Artemia* populations to elevated salinities. It is furthermore our intention to check if the inoculated *Artemia* strain is better adapted to high salinities than the local parthenogenetic population, and therefore may control in a more effective way eutrophication caused by algae, especially in high salinities.

Materials and methods

The two *Artemia* populations evaluated were the parthenogenetic population *A. parthenogenetica* from the man-made salterns near the city of Tanggu, Tianjin province, People's Republic of China (TG; material collected in April 1991) and the bisexual *Artemia franciscana* from Leslie saltworks in the San Francisco Bay, California, USA (SFB; cysts obtained from the San Francisco Bay Brand Inc., batch No 2891).

Culture experiments

Cysts were incubated in artificial 0.45 μm-filtered 35 ppt Dietrich & Kalle (D&K) (1963 – in Kinne, 1971) medium for hatching under optimal conditions (Sorgeloos *et al.*, 1986).

Two culture experiments were performed for studying the effect of salinity on survival, time to maturity and growth rate. In the first experiment the salinity was gradually increased from 35 to 60, 100, 140 or 180 ppt in such a way that animals reached the salinity of 180 ppt after 16 days of culture by daily steps of 10 to 15 ppt by replacing part of the culture water with D&K medium of higher salinity. As soon as the salinity reached the desired level, it was kept constant for the rest of the experiment. In the second experiment, instar-I nauplii were transferred directly to the five selected salinities and acclimatized for 24 hrs while food was added. Then, the surviving nauplii were counted and transferred to cylindroconical tubes. The salinity of the freshly-prepared D&K medium was 120 ppt. The desired salinities were obtained either by evaporating the water in shallow plastic tanks at 30 °C, or by adding deionized water.

Table 1. Feeding schedule, in 10^6 cells per day, for 200 individuals (modified from Coutteau *et al.*, 1992).

Day of culture	*Dunaliella tertiolecta*	Lansy PZ
1	7.51	67.63
2, 3, 4	8.91	80.19
5, 6	13.50	121.50
7	17.82	160.38
8	37.80	340.20
9	61.20	550.80
10, 11	72.00	648.00
12, 13	90.00	810.00
14, 15	108.00	972.00
16, 17	126.00	1,134.00
18, 19	153.00	1,377.00
20 and afterwards	180.00	1,620.00

In both experiments the initial number of nauplii per tube was 400. Initial animal density was one nauplius per 2 ml of cultured medium, reduced after day 8 to one individual per 3 ml, while from day 14 onwards the density was kept at one animal per 4 ml. All experiments were carried out at 25±1 °C with 12 hr cool white fluorescent lighting daily. Aeration was applied from the bottom and the tubes were covered with perforated Petri dishes to minimize evaporation. The experiments were run in triplicate and the animals were fed on a mixed diet of the alga *Dunaliella tertiolecta* Butch and the yeast-based formulated feed LANSY PZ (from Artemia Systems SA, Belgium) (Coutteau, 1992; Coutteau *et al.*, 1992), following the feeding schedule given in Table 1.

Survival and maturation rates were determined at each water renewal *i.e.* on day 8, 11, 14, 17, 20 and 23, (females were considered mature when migration of the oocytes into the uterus was observed). The results (expressed as percentage) were arcsine transformed and analyzed by a standard single factor ANOVA (Sokal & Rohlf, 1981).

Biometry

On days 8, 14, 20 and 26 a random sample of at least 10 animals per cone were examined (at least 30 individuals per salinity) for the following morpholog-

ical parameters: total length, abdominal length, length from the end of the 8th abdominal segment to the third abdominal segment, length of the eighth abdominal segment, width of the head, abdominal width, and length of the first antenna. The animals were anaesthetized in chloroform-saturated seawater (Gilchrist, 1960) and measured under a dissection microscope equipped with a camera lucida and then the data was analyzed with a digitizer.

The results were processed with discriminant function analysis (Pimentel, 1979; Sokal & Rohlf, 1981; Hontoria & Amat, 1992a, b); this multivariate procedure computes a series of new variables (Z_1, Z_2, ...) which are linear functions of the morphological parameters considered with the form $Z_n = l_1X_1 + l_2X_2 + ...$ (where l_n are the calculated discriminant coefficients and X_s the variables being considered). This discriminant function is constructed in such a way that as many as possible members of one population have high values for Z and as many as possible members of the other have low values, so that Z serves as a much better discriminant of the two populations than variable X_1 or X_2 does taken singly (for more details see Pimentel, 1979; Sokal & Rohlf, 1981). The calculations were performed with the statistical package Statgraphics version 5.0 (Statistical Graphics Corp., Rockville, MD-USA).

Reproductive and lifespan characteristics

The reproductive performance of the two populations was tested at the five selected salinities and at 25±1 °C. Nauplii were transferred to the different salinities directly, as with the second experiment. As soon as males started to clasp females (in the case of the dioecious population SFB) or when the parthenogenetic females showed signs of ovarian development, couples or parthenogenetic females were removed from the mass culture and placed in 50 ml plastic cylindroconical tubes.

Twenty females from the parthenogenetic population were examined for each salinity. Each female was placed in a tube with 25 ml of 0.45 μm filtered D&K of the appropriate salinity. For SFB, each pair (20 pairs per salinity) was placed in a 50 ml tube. Males were replaced at death or if female reproduction ceased (Browne *et al.*, 1988). The tubes were examined daily for offspring production or deaths. Reproductive and life span characteristics were determined according to Browne *et al.* (1984; 1988).

The results were analyzed by a standard single factor ANOVA, where variances are assumed to be homogeneous (Sokal & Rohlf, 1981). However, tests for homogeneity of variances (Bartlett's test and Hartley's Fmax-test) showed significant deviations from equal variances for most of the studied characteristics. Since log or square-root transformation did not efface the problem, we conducted an approximate test of equality of means when variances were assumed to be unequal and sample sizes varied (Games & Howell, 1976).

Results

Survival

The survival of the two populations for the two different experiments is presented in Figs 1 and 2. Salinity effects on survival after 23 days were statistically analyzed and the results revealed that the bisexual population has significantly higher survival in a broader range of salinities (ANOVA. $P<0.05$). Direct transfer of nauplii to high salinities had negative effect in both populations, particularly at 180 ppt, where both species exhibited less than 20% survival after 23 days of culture. However, gradual increase of the salinity resulted in a longer survival of *A. franciscana* compared to *A. parthenogenetica*. After 23 days of gradual increase in salinity (Fig. 1), the survival of the SFB at 180 ppt was 69.44% while on the same day TG had a much lower performance (14.54%; ANOVA, $P<0.05$). The parthenogenetic population did well at salinities of 60 and 100 ppt while at 35, 140 and 180 ppt the survival was less than 50% after 23 days of culture ($P<0.05$). At 60 and 100 ppt, after gradual increase of the salinity SFB is not significantly different from TG (ANOVA, $P>0.05$), but at 35, 140 and 180 ppt the survival for SFB is much higher (ANOVA, $P<0.05$).

Maturity

The results from the two experiments are presented in Table 2. The two higher salinities used in this study significantly affect the period that is required to reach maturity (ANOVA $P<0.05$). The maturation period is shorter when the animals are gradually acclimatized. On day 23 of the first experiment, almost all salinities examined gave high numbers of mature animals for the TG population except for the 180 ppt salinity which yielded 60.17%. A direct transfer of parthenogenetic nauplii to high salinities significantly affected

Table 2. Mean (± s.d.) *Artemia* maturity for days 17, 20, 23 and 26, after a gradual increase of the salinity (experiment-1) and after a direct transfer to higher salinities (experiment-2). $^{a,b,\ldots k}$ values in each column sharing the same letter are not significantly different (Anova, $P<0.05$); values represent the mean of three replicates per salinity.

Day of culture	Salinity (In ppt)	Average percentage of maturation							
		Tanggu				SFB			
		Experiment-1		Experiment-2		Experiment-1		Experiment-2	
DAY 17	180	$12.92^{a,b}$	(6.32)	0.00^{a}	(0.00)	11.32^{b}	(1.68)	0.00^{a}	(0.00)
	140	8.79^{a}	(3.84)	0.00^{a}	(0.00)	7.14^{ab}	(2.19)	6.84^{b}	(2.32)
	100	14.03^{ab}	(6.48)	30.22^{c}	(3.63)	9.02^{ab}	(3.02)	21.44^{cd}	(3.01)
	60	20.16^{b}	(2.29)	38.52^{cd}	(24.63)	7.82^{ab}	(1.44)	11.81^{bc}	(6.36)
	35	23.24^{b}	(10.58)	12.80^{b}	(5.00)	4.24^{a}	(0.98)	5.57^{b}	(4.65)
DAY 20	180	63.70^{c}	(0.49)	0.00^{a}	(0.00)	41.41^{c}	(15.54)	1.06^{a}	(1.51)
	140	88.47^{d}	(14.31)	8.39^{b}	(5.94)	60.14^{de}	(14.31)	30.90^{de}	(2.37)
	100	98.84^{ef}	(0.65)	83.15^{fg}	(4.32)	64.24^{def}	(0.65)	51.90^{fg}	(8.67)
	60	93.34^{de}	(10.48)	88.45^{g}	(4.69)	69.84^{efg}	(10.48)	54.27^{g}	(2.00)
	35	96.89^{de}	(0.40)	65.18^{e}	(6.00)	55.37^{d}	(0.40)	39.40^{ef}	(5.00)
Day 23	180	60.17^{c}	(0.43)	0.00^{a}	(0.00)	63.67^{def}	(9.62)	9.65^{b}	(4.76)
	140	99.80^{f}	(0.35)	45.83^{d}	(8.68)	80.54^{gh}	(3.94)	68.01^{h}	(14.03)
	100	99.34^{ef}	(0.58)	97.17^{h}	(3.86)	78.94^{gh}	(5.62)	82.84^{ijk}	(6.44)
	60	96.41^{de}	(1.02)	98.87^{hi}	(0.50)	72.09^{efg}	(11.84)	64.17^{gh}	(2.65)
	35	99.60^{ef}	(0.39)	99.07^{hi}	(0.89)	63.00^{def}	(6.00)	53.69^{g}	(5.62)
DAY 26	180	68.17^{c}	(2.00)	0.00^{a}	(0.00)	73.56^{fg}	(5.32)	35.36^{de}	(14.59)
	140	100.00^{f}	(0.00)	75.33^{ef}	(6.42)	88.41^{hi}	(2.10)	84.20^{jk}	(1.24)
	100	100.00^{f}	(0.00)	100.00^{i}	(0.00)	93.29^{ij}	(4.37)	89.34^{k}	(1.41)
	60	100.00^{f}	(0.00)	100.00^{i}	(0.00)	95.54^{j}	(2.10)	75.37^{hij}	(6.76)
	35	100.00^{f}	(0.00)	100.00^{i}	(0.00)	92.02^{ij}	(2.30)	72.79^{hi}	(4.93)

the development (ANOVA, $P<0.05$), resulting in few mature individuals. In contrast, when SFB strain was reared at high salinity, it resulted in a high number of mature animals, although the period needed to reach maturity was observed to be longer.

Statistical analysis (ANOVA, $P<0.05$) revealed that for both species the salinity of 180 ppt caused significant delay in their maturation period compared to that observed at lower salinity levels.

Growth

Growth rates are presented in Figs 3 and 4. Salinity had no significant effect upon TG population during the first experiment (Fig. 3), while the SFB population exhibited statistically significant differences between low and high salinities (see Fig. 3), with higher growth rates at lower salinities.

In the second experiment (Fig. 4), the salinity of 180 ppt caused significantly slower growth (ANOVA, $P<0.05$) of parthenogenetic individuals (5.27 mm total length after 26 days). In the case of the bisexual population, the best performance was recorded at the low salinities (35 ppt), while after 26 days of culture no striking differences were observed at salinities of 100, 140 and 180 ppt (ANOVA, $P<0.05$). The males constantly appeared to be smaller than the bisexual females.

Biometry

Tables 3 and 4 summarize the mean values of the variables measured on female and male brine shrimp on day 26 of the second experiment. All the animals considered were adults, except for the TG 180 ppt population where no adults were present. The results of the discriminant analysis are presented in Table 5. Discriminant analysis, based on the 'type' of population (parthenogenetic or bisexual) as the separation factor, revealed one discriminant function for each salinity and always gave 100% prediction for day 26 and very high prediction and discrimination for days 20 and 14

Table 3. Mean (± s.d.) of various morphometric parameters of female *Artemia* cultured under standard laboratory conditions. The results are from day 26 of the second experiment. A: total length, B: abdominal length, C: length from the end of the 8th abdominal segment to the 3rd abdominal segment, D: length of 8th abdominal segment, E: width of head, F: abdominal width, G: length of first antenna, H: per cent of abdomen length/total length (TL), I: per cent C/TL, J: per cent D/TL, K: per cent E/TL, L: per cent F/TL, M: per cent G/TL.

	Tanggu					SFB				
	35	60	100	140	180	35	60	100	140	180
A	10.31	10.57	10.46	8.86	5.27	10.16	9.34	8.27	7.56	6.37
	(0.74)	(0.92)	(0.85)	(0.07)	(0.99)	(0.85)	(1.13)	(0.84)	(0.26)	(0.42)
B	5.38	5.41	5.46	4.64	2.81	4.65	4.33	3.89	3.38	3.17
	(0.39)	(0.54)	(0.50)	(0.12)	(0.67)	(0.57)	(0.64)	(0.50)	(0.14)	(0.27)
C	4.09	4.18	4.18	3.65	2.13	3.42	3.22	3.02	2.59	2.38
	(0.29)	(0.42)	(0.39)	(0.09)	(0.46)	(0.37)	(0.56)	(0.43)	(0.08)	(0.23)
D	1.16	1.15	1.13	0.92	0.65	0.98	0.95	0.84	0.78	0.70
	(0.09)	(0.12)	(0.11)	(0.06)	(0.12)	(0.13)	(0.15)	(0.15)	(0.02)	(0.07)
E	1.56	1.53	1.42	1.24	0.67	1.17	1.62	1.55	1.32	1.03
	(0.09)	(0.17)	(0.16)	(0.04)	(0.16)	(0.14)	(0.16)	(0.15)	(0.04)	(0.04)
F	0.64	0.61	0.60	0.57	0.30	0.84	0.79	0.75	0.62	0.48
	(0.05)	(0.05)	(0.05)	(0.01)	(0.04)	(0.07)	(0.07)	(0.10)	(0.02)	(0.02)
G	1.11	1.12	1.03	0.97	0.46	0.70	0.67	0.70	0.74	0.58
	(0.09)	(0.08)	(0.09)	(0.12)	(0.14)	(0.08)	(0.07)	(0.09)	(0.01)	(0.05)
H	51.49	51.19	52.14	51.77	48.43	45.63	42.27	46.52	44.44	47.19
	(1.68)	(1.68)	(1.67)	(0.14)	(3.36)	(2.26)	(2.49)	(3.36)	(0.99)	(1.41)
I	39.15	39.53	39.92	40.76	40.32	33.60	34.36	36.42	34.24	36.97
	(1.45)	(1.53)	(1.69)	(0.20)	(2.53)	(1.69)	(3.06)	(3.17)	(0.08)	(1.20)
J	11.12	10.85	10.77	10.33	12.12	9.61	10.91	10.21	10.30	10.88
	(0.54)	(0.61)	(0.69)	(0.90)	(0.94)	(0.94)	(1.27)	(1.76)	(0.38)	(0.26)
K	15.18	14.48	13.65	13.82	12.65	16.89	17.50	18.86	17.52	16.36
	(1.21)	(1.07)	(1.43)	(0.73)	(0.82)	(0.99)	(1.93)	(1.80)	(0.11)	(0.31)
L	6.16	5.78	5.75	6.36	5.86	8.31	8.32	9.08	8.18	7.70
	(0.50)	(0.36)	(0.50)	(0.06)	(0.50)	(0.66)	(0.83)	(1.09)	(0.23)	(0.21)
M	10.87	10.65	9.89	10.82	8.68	6.89	7.29	8.55	9.83	9.17
	(1.30)	(0.87)	(0.94)	(0.24)	(1.17)	(0.60)	(1.09)	(1.22)	(0.37)	(0.15)

(100% for all salinities except for 180 ppt which was ≥75%). The morphological parameters which contributed mostly to the discrimination between the two populations are: the length of first antenna for low salinities and the width of head for higher salinities. These two characteristics allowed us to discriminate between the two species on day 26 and gave 100% separation in all cases except for 140 ppt salinity where the discrimination was 89.29% for TG and 94.12% for SFB.

Reproductive and life span characteristics

Six reproductive and four lifespan characteristics from the two populations cultured at five different salinities are summarized in Table 6. Statistical analysis using an approximate test of equality of means (Games & Howell, 1976) indicated that significant differences exist between the two populations in most of the characteristics studied. A summary of the statistics is presented in Table 7.

Interpopulation comparison of the reproductive and lifespan characteristics revealed that the bisexual and the parthenogenetic population are significantly different at 35, 60 and 100 ppt (with greater values of the SFB), while at 140 and 180 ppt there are no significant differences for the majority of the characteristics studied. At all salinities except 100 ppt, SFB produced a higher percentage of encysted embryos than TG. At the

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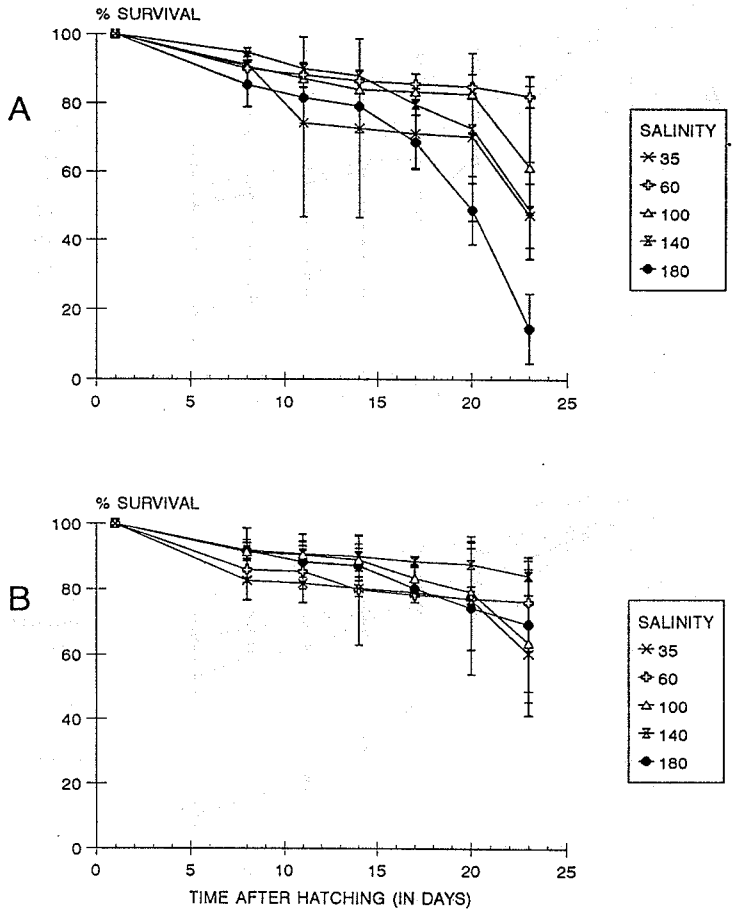

Fig. 1. Survival of *Artemia* after gradual increase of the salinity. A: *Artemia parthenogenetica*. B: *Artemia franciscana*.

intrapopulation level, SFB produced a higher percentage in the extreme salinities (35, 140 and 180 ppt).

Intrapopulation comparison revealed that salinity above 140 ppt has a negative effect on reproductive output. This is most obvious when considering the number of offspring per brood and the number of broods per female (especially for SFB). As a result, highly significant differences ($P<0.01$) exist in the case of the bisexual population in terms of total offspring per female, with a major decrease in reproductive output at the two highest salinities.

Life span characteristics are also affected by salinity. High salinities cause delay in development (e.g. at low salinities first reproduction occurred approximately in 22 days, while this period was longer at 180 ppt). In both species the prereproductive period is significantly longer at 180 ppt. SFB has the longest reproductive period at 100 ppt, and this period is in general longer than it is in TG. Postreproductive period was not significantly different except for the SFB at 140 ppt. In most cases the life span of SFB was longer than that of TG.

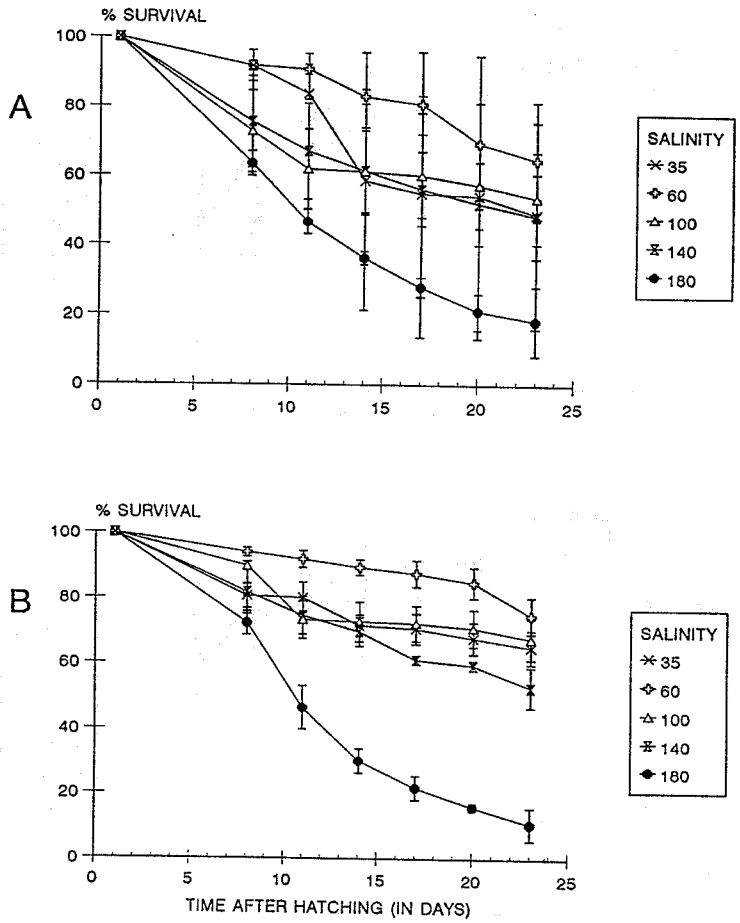

Fig. 2. Survival of *Artemia* after direct transfer of 24 hr acclimated larvae to different salinities. A: *Artemia parthenogenetica*. B: *Artemia franciscana*.

Standard deviations in the parthenogenetic population in most cases were smaller than those in the bisexual.

Discussion

The two populations studied exhibit significant differences in their life strategies and responses under various salinity regimes. Marked differences occur at intrapopulation as well as interpopulation level.

Survival is affected by salinity and both species experienced high mortality when nauplii were directly transferred to elevated salinities. However, when salinity is gradually increased, *A. franciscana* exhibits better adaptation. Gradual increase of salinity has no beneficial effect upon the TG, *i.e.* at salinities above 140 ppt the mortality is high and the survivors display retarded growth and poor reproductive characteristics.

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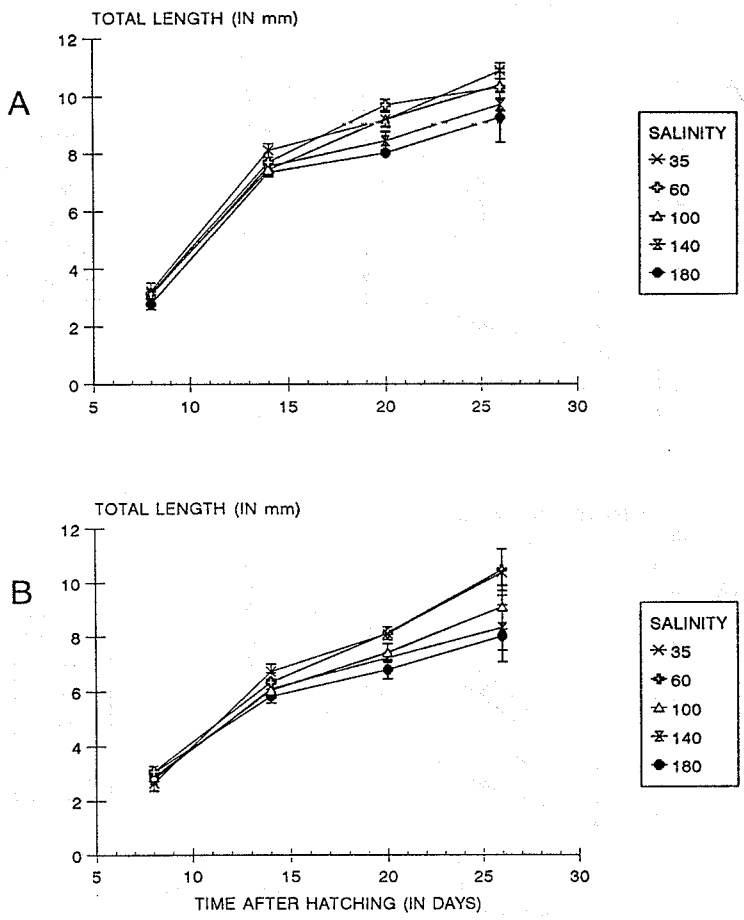

Fig. 3. Growth of *Artemia* in five salinities at 25 °C. The salinity was gradually increased with daily steps of 10 to 15 ppt until the desired level is reached. A: *Artemia parthenogenetica*. B: *Artemia franciscana*.

This confirms the on-site observations of Tackaert & Sorgeloos (1991) that the parthenogenetic population from TG experiences high mortality at high salinities.

Brine shrimp nauplii can resist sudden shifts in salinity, e.g. instar I nauplii may be transferred from 5 ppt to 150 ppt without increased mortality (D'Agostino & Provasoli, 1968; Sorgeloos, 1980). Although it is well known that *Artemia* in its natural environment can stand salinities higher than 200 ppt (Persoone & Sorgeloos, 1980), there are always problems in maintaining *Artemia* at salinities higher than 200 ppt in the laboratory (Wear & Haslett, 1986; Wear *et al.*, 1986). Browne & Hoopes (1990) reported high mortality in a parthenogenetic population from Salin de Giraud (France) at a salinity above 150 ppt (9% survival at 190 ppt) while at 230 ppt all individuals died before day 10. Dana & Lenz (1986) studying the bisexual *Artemia* from Mono Lake, California, report-

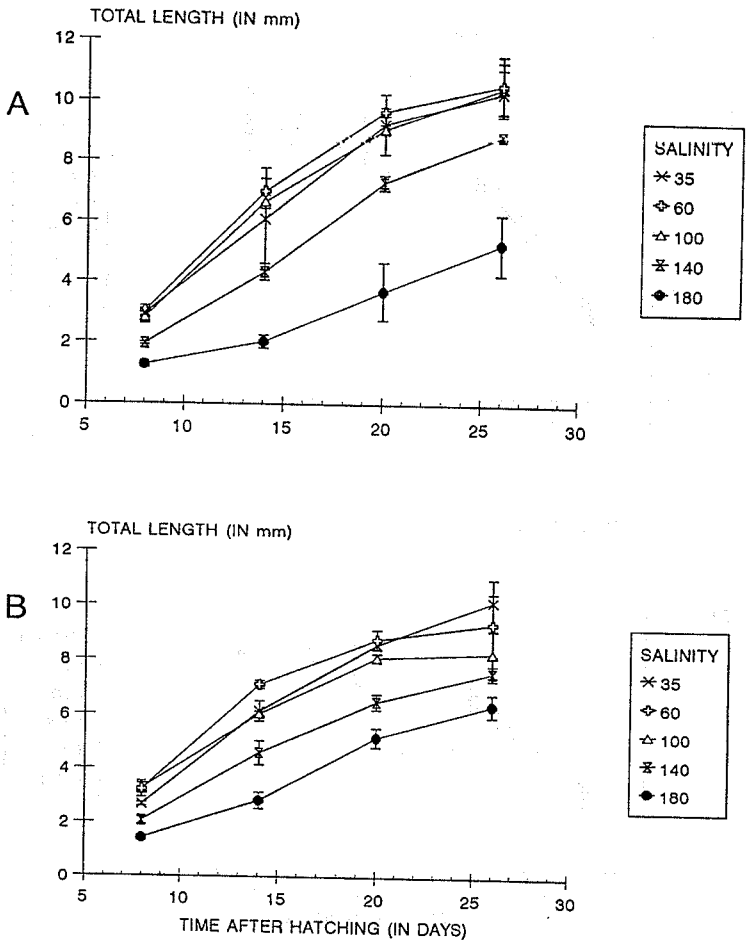

Fig. 4. Growth of *Artemia* in five salinities at 25 °C. Animals were transferred directly to the five salinities after 24 hr acclimation in D&K medium. A: *Artemia parthenogenetica*. B: *Artemia franciscana*.

ed low survival in salinities of 159 and 179 ppt under laboratory conditions.

The present study shows that the optimum salinity for the TG parthenogenetic population is between 60 and 100 ppt while *A. franciscana* (as represented by SFB) appears to be more euryhaline, exhibiting high survival and better reproductive characteristics in a broader range of salinities (Wear & Haslett, 1986; Wear *et al.*, 1986). The parthenogenetic population presents relatively low survival at the salinity of 35 ppt (less than 50% in both experiments after 23 days of culture) confirming the observations of Vanhaecke *et al.* (1984).

In accordance with Bond (1933), Gilchrist (1960) and Dana & Lenz (1986) adult size, maturation and growth rates were inversely proportional to salinity, especially above 140 ppt. Data for growth rates in combination with data for length of the prereproductive periods provide evidence that high salinities restrain colonization of the saltworks, namely, that it can take a

Table 4. Mean (± s.d.) of various morphometric parameters of male *Artemia franciscana* cultured under standard laboratory conditions. The results are from day 26 of the second experiment. A: total length, B: abdominal length, C: length from the eighth abdominal segment to the third abdominal segment, D: length of the eighth abdominal segment, E: width of head, G: length of first antenna, H: per cent of abdomen length/total length (TL), I: per cent C/TL, J: per cent D/TL, K: per cent E/TL, M: per cent G/TL.

Parameters	Salinity (ppt)				
	35	60	100	140	180
A	8.36	7.91	6.91	6.92	5.47
	(0.75)	(0.71)	(0.62)	(0.23)	(0.24)
B	3.57	3.53	3.01	3.17	2.43
	(0.11)	(0.22)	(0.10)	(0.05)	(0.16)
C	2.92	2.78	2.34	2.46	1.99
	(0.28)	(0.30)	(0.27)	(0.06)	(0.14)
D	0.88	0.82	0.76	0.79	0.62
	(0.10)	(0.09)	(0.07)	(0.05)	(0.09)
E	1.88	1.71	1.73	1.58	1.17
	(0.15)	(0.17)	(0.16)	(0.04)	(0.02)
G	1.15	1.07	1.08	1.10	0.79
	(0.15)	(0.12)	(0.06)	(0.03)	(0.01)
H	43.19	43.69	43.55	44.23	43.72
	(0.50)	(1.43)	(0.50)	(0.37)	(1.30)
I	34.95	35.08	33.89	35.15	36.07
	(2.16)	(1.81)	(3.31)	(0.24)	(1.04)
J	10.61	10.30	11.04	11.24	11.54
	(1.24)	(0.69)	(1.16)	(0.53)	(1.02)
K	22.60	21.67	24.95	23.15	21.78
	(1.53)	(1.11)	(1.89)	(0.19)	(1.06)
M	13.75	13.53	15.59	16.03	14.80
	(1.58)	(1.20)	(1.38)	(0.68)	(0.65)

period of 30 days (or even more in the case of 180 ppt) before the first offspring are released. During water circulation in the saltworks, great care should be taken to ensure that the population of *Artemia* can increase as soon as possible under optimal salinity conditions, taking advantage of the beneficial filter-feeding activity of this crustacean.

Reeve (1963) observed a maximal growth rate in Great Salt Lake *A. franciscana* at a salinity of 35 ppt. This is in agreement with our findings of increased growth rate of *A. franciscana* from San Francisco Bay at salinities between 35 and 60 ppt (see Fig. 4b and Table 3). However, TG *A. parthenogenetica* display growth retardation at salinities of 140 and 180 ppt (see Fig. 4a and Table 3). This retardation would probably be more pronounced at spring temperatures of 10 ° to 15 °C (Tackaert & Sorgeloos, 1991) which prevail in the Tanggu saltworks.

In the case of a mixed population consisting of bisexual and parthenogenetic individuals, the discrimination method proposed by Hontoria & Amat (1992a, b) could be a useful tool for distinction of the two groups and their distribution in the ponds. Our study on morphometric parameters of adult and preadult *Artemia* revealed that the length of first antenna is the best characteristic for identification of parthenogenetic individuals. The first antenna is significantly longer in parthenogenetic *Artemia* from Tanggu than in *A. franciscana*. The differences in morphological characteristics between the two populations allow the use of discriminant analysis to allocate individuals to the correct species as of day 14 with almost 100% accuracy. Biometry can be used to estimate the participation of each species in a mixed population and, therefore, to be of help to the managerial procedure of a saltwork.

The results presented in this study indicate that salinity has a marked effect on the life cycle characteristics of both species examined. In terms of reproductive output and life span, SFB has higher values at most of the salinities studied than TG females. No significant differences have been observed at 180 ppt (except for offspring encysted). This might be attributed to the fact that only seven females from the parthenogenetic population were examined (the only survivors) and thus selective pressure might have been very high, allowing only the most adapted genotypes to survive in such extreme environmental conditions.

An important difference in the 180 ppt treatment is the percent of encysted offspring which is significantly higher for the SFB bisexual population (row E, Table 6). Interestingly, production of cysts by SFB was much higher than that from TG in all salinities examined except for 100 ppt. However, cyst production seems to be more favored in the salinities of 35, 140 and 180 ppt.

The high standard deviations observed for reproductive and lifespan characteristics of the bisexual population presumably reflect high heterogeneity and intrapopulation variance; in contrast, the variance levels for the parthenogenetic population are much lower, as expected since the genotypic diversity in bisexual species is higher than that of parthenogenetic populations. However, the standard deviations for the TG parthenogenetic population are higher than those reported by Browne & Hoopes (1990) for the clones of a parthenogenetic population from Salin de Giraud

Table 5. Discriminant analysis of morphometric variables measured in *Artemia* females on day 26 of the second experiment. Unstandardized coefficients from the discriminant function are presented as well as group centroids and prediction group. 100 per cent prediction means that with the variables used, 100% discrimination of the two species can be obtained. A: total length, B: abdominal length, C: length from the end of the eighth abdominal segment to the third abdominal segment, D: length of the eighth abdominal segment, E: width of head, F: abdominal width, G: length of first antenna.

Variables	Discriminant function coefficients for the various salinities				
	35 ppt	60 ppt	100 ppt	140 ppt	180 ppt
A	− 1.31081	0.21540	0.21891	− 1.04617	− 1.04928
B	1.43216	− 2.11203	1.95454	3.42127	8.20983
C	1.77898	2.43352	− 1.49819	1.45677	− 6.83560
D	− 0.78390	− 0.13884	1.04606	0.96227	3.04348
E	− 0.29192	0.30999	− 4.01178	− 1.59466	− 10.4906
F	− 10.1299	14.84500	− 7.68642	− 17.2585	− 22.3799
G	9.56614	− 13.07470	9.49617	2.11878	8.27474
Constant	0.61149	0.64627	− 4.16915	− 0.61296	12.2272
Group centroids					
TANGGU	4.67940	− 3.68043	3.17926	2.28659	4.43767
SFB	− 3.11960	4.85147	− 3.81511	− 3.76615	− 1.38677
Predicted group					
TANGGU	100%	100%	100%	100%	100%
SFB	100%	100%	100%	100%	100%

(France), as well as for other parthenogenetic populations (Browne *et al.* 1984). Although this might be due to differences in methodology, it could also indicate that the TG population is characterized by higher multiclonality as it was observed in other polyploid parthenogenetic *Artemia* populations (Abatzopoulos *et al.*, 1986, 1993). The latter hypothesis is the most likely as it appears from Zhang *et al.*, (1991) and personal observations (unpublished data) that TG consists of a mixture of diploid, tetraploid and pentaploid individuals.

Conclusion

The data derived from the study of reproductive and lifespan characteristics of SFB and TG populations provide evidence that the TG parthenogenetic population is poorly adapted to high salinities and therefore can not yield large populations, while the SFB strain seems to be a more effective colonizer at elevated salinities.

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Table 6. Mean (± s.d.) of reproductive and lifespan characteristics for the TG and SFB populations reared in five salinities. A: offspring per brood, B: broods per female, C: offspring per female per day, during the reproductive period, D: days between broods, E: percent offspring encysted, F: total offspring per female, G: prereproductive period (in days), H: reproductive period (in days), I: postreproductive period (in days), J: total life span (in days). N=20 in all cases except TG 180 ppt where N=7.

	35 ppt		60 ppt		100 ppt		140 ppt		180 ppt	
	TANGGU	SFB	TANGGU	SFB	TANGGU	SFB	TANGGU	SFB	TANGGU	SFB
A	64.07	89.63	67.04	87.68	66.17	86.83	44.10	45.36	7.52	11.26
	(13.25)	(29.97)	(12.02)	(36.52)	(13.31)	(21.16)	(13.55)	(23.64)	(11.94)	(13.36)
B	4.60	10.20	2.80	7.89	5.20	14.65	3.50	4.15	1.71	1.50
	(2.62)	(7.44)	(2.25)	(7.39)	(3.28)	(6.78)	(3.06)	(3.64)	(2.91)	(2.13)
C	19.41	31.96	23.02	31.95	17.96	23.04	16.16	11.48	1.26	4.39
	(13.98)	(34.24)	(15.43)	(19.75)	(13.50)	(8.12)	(13.00)	(10.82)	(2.13)	(8.66)
D	4.71	3.87	4.71	3.67	4.64	3.61	4.82	8.73	7.12	2.76
	(0.76)	(0.79)	(0.61)	(0.79)	(0.99)	(0.77)	(1.66)	(8.29)	(2.55)	(4.85)
E	16.92	49.91	13.03	38.53	7.79	15.60	18.99	52.67	4.94	41.24
	(12.95)	(30.31)	(10.22)	(31.77)	(8.49)	(21.20)	(17.95)	(35.30)	(9.03)	(33.29)
F	297.30	882.25	185.95	671.00	331.20	1,348.00	147.90	227.40	46.29	37.45
	(297.30)	(655.05)	(148.12)	(624.43)	(203.24)	(753.17)	(121.81)	(209.03)	(81.01)	(55.52)
G	21.15	22.85	20.70	20.75	20.60	20.30	24.35	34.40	41.14	43.60
	(0.91)	(3.18)	(1.65)	(1.34)	(0.92)	(2.10)	(1.88)	(14.99)	(6.33)	(15.62)
H	21.35	43.15	11.40	33.84	24.05	62.00	15.70	34.44	10.14	10.75
	(14.46)	(33.93)	(12.76)	(36.12)	(18.64)	(29.06)	(16.84)	(29.71)	(16.15)	(17.61)
I	1.40	1.20	1.30	2.21	3.65	2.30	2.65	5.11	0.57	1.90
	(2.73)	(1.50)	(1.55)	(2.14)	(4.23)	(1.79)	(4.42)	(6.75)	(1.05)	(4.64)
J	43.90	67.60	33.40	56.89	48.30	85.60	42.70	70.00	51.86	54.75
	(14.27)	(32.76)	(13.95)	(37.35)	(17.09)	(29.55)	(19.47)	(29.83)	(19.68)	(27.08)

Table 7. Significant differences as determined by the Games and Howell (1976) method ($P<0.05$) for reproductive and lifespan characteristics of TG and SFB populations reared at different salinities. Populations (for each characteristic) that share the same letter (a, b,..., d) are not significantly different. A: offspring per brood, B: broods per female, C: offspring per female per day, during the reproductive period, D: days between broods, E: percent offspring encysted, F: total offspring per female, G: prereproductive period (in days), H: reproductive period (in days), I: postreproductive period (in days), J: total life span (in days).

Population	Salinity	A	B	C	D	E	F	G	H	I	J
TG	35	b c	b	b c	a b	a	b	a	a b	a b c	a b
SFB	35	d	d	d	a b	b	c	a	c	a b	c
TG	60	c	ab	cd	ab	a	ab	a	a	ab	a
SFB	60	d	c d	d	a b	b	c	a	bc	a b c	b c
TG	100	c	b c	b c	ab	a	b	a	ab	a cd	ab
SFB	100	d	d	cd	a b	a	c	a	d	a b c	d
TG	140	b	a b	b c	b	a	a b	a	a	a b c	a b
SFB	140	a b	a b	a b	c	b	a b	b	b c	d	c d
TG	180	a	a b	a	a b c	a	a b	c	a	a	a b c
SFB	180	a	a	a	a	b	a	c	a	a b c	b c

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